REMARKS

Claims 1-13 are pending in the present application. Claims 1, 2 and 6-13 are rejected, and claims 3-5 are objected to. Upon entry of the instant Amendment, claims 3 to 9, 11 and 12 will be pending, and claims 1 and 2 will be cancelled.

Election/Restrictions

The Applicants thank the Examiner for having examined the new group of species from claim 9 of "Sterilization process utilizing chemicals" and species listed in claim 10.

Rejection under 35 USC § 112

Claim 9 is allegedly unclear under USC § 112, second paragraph, because of the term "alkylant". Although the Applicants consider that this term is known in the art, it is herein changed to read "alkylating" in order to accelerate prosecution.

Claims 2 and 6 are rejected under 35 USC § 112, first paragraph. Claim 2 is cancelled, rendering this rejection moot. Claim 6 is herein amended to depend on claim 3 which now recites that the prion protein degradation indicator is transcribed by a gene naturally occurring in a fungus selected from the group consisting of *Saccharomyces cerevisiae* and *Podospora anserine*. Such was previously recited in claims 1 and 2. Support can be found throughout the specification, for example at page 3, lines 30-34, and page 5, lines 24-31.

Applicants respectfully submit that the classical definition of a prion, as shown in E. D. Ross *et al., Nature Cell Biology*, 2005, 7(11):1039-1044, does not include Aβ protein. More

specifically, in the first sentence of the abstract, it is stated that prions are infectious proteins and are self-propagating, which is not the case of the Aβ protein. Furthermore, Applicants submit that Aβ protein, responsible for forming amyloid plaques in Alzheimer disease, is not found in fungi. Applicants respectfully submit that the identification of new prion proteins in fungi is not unpredictable and is supported in the application as filed.

Rejection under 35 USC § 102

Claims 1, 6, 7 and 9 are rejected under 35 U.S.C. 102(b) as being allegedly anticipated by Reichl. Claims 1 and 6 to 12 are rejected under 35 U.S.C. 102(b) as being allegedly anticipated by Safar *et al.* The Examiner acknowledges that claims 3 to 5 would be allowable if rewritten in independent form, including all of the limitations of the base claim and any intervening claim.

Claim 1 is cancelled herein purely in the interest of advancing prosecution. Claims 3 to 5 are rewritten in independent form, including all of the limitations of the base claim and any intervening claims. Claims 6 to 12 are dependent upon claim 3. Hence, all of the claims, including claims 6 to 12 are patentable over Reichl and Safar *et al.*

Rejection under 35 USC § 103

Claim 9 is rejected under 35 U.S.C. 103(a) as allegedly unpatentable over Safar *et al.* in view of Feldman *et al.* Claims 9, 10 and 13 are rejected under 35 U.S.C. 103(a) as allegedly unpatentable over Safar *et al.* in view of Dresdner Jr. *et al.* The Examiner acknowledges that claims 3 to 5 would be allowable if rewritten in independent form, including all of the limitations of the base claim and any intervening claim.

Claims 1 and 2 are cancelled herein, claims 3 to 5 are rewritten in an independent form, including all of the limitations of the base claim and any intervening claim, and claims 9, 10 and 13 are dependent on claim 3. Applicants respectfully submit that the instant prion protein degradation indicator is conceived for clinical use in health institutions. The prior art describes indicators that are not transposable in health institutions, because they require use in either specialized research laboratories or confined areas that are absolutely not available in a central sterilization room as found

in an hospital. Hence, Applicants submit that claims 9, 10 and 13 are neither taught nor suggested by Safar in view of Feldman *et al.*, nor by Safar in view of Dresdner.

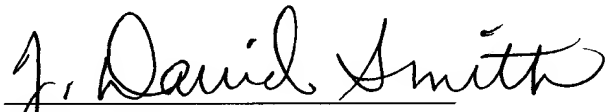
FEES

No additional fees are believed to be necessary. However, if any additional fees are due, authorization is hereby given to charge Deposit Account No. 11-1153 for any underpayment.

CONCLUSION

Applicants respectfully request entry of the foregoing amendments and remarks in the file history of the instant Application. Early and favorable action on the claims is earnestly solicited. If any issues may be resolved by telephone, the Examiner is invited to contact the undersigned at the telephone number provided below.

Respectfully submitted,



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Prion domains: sequences, structures and interactions

Eric D. Ross, Allen Minton and Reed B. Wickner

Mammalian and most fungal infectious proteins (also known as prions) are self-propagating amyloid, a filamentous β-sheet structure. A prion domain determines the infectious properties of a protein by forming the core of the amyloid. We compare the properties of known prion domains and their interactions with the remainder of the protein and with chaperones. Ure2p and Sup35p, two yeast prion proteins, can still form prions when the prion domains are shuffled, indicating a parallel in-register β-sheet structure.

In the decades since prions were proposed to be responsible for mammalian transmissible spongiform encephalopathies (TSEs, such as scrapie of sheep and Creutzfeldt–Jakob disease (CJD) of humans)[1,2], significant progress has been made in understanding these diseases. However, investigation of *de novo* TSE generation is precluded by its rarity, and extraordinarily long incubation times make even studies of propagation difficult. Prions of yeast and other fungi offer many experimental advantages in examining fundamental questions of prion biology and of the wider amyloid field. There are many excellent and comprehensive reviews of yeast and human prions[3–6]; here, we focus on the prion domains of the amyloid-based prions.

Amyloid is a filamentous protein form that is characterized by increased protease resistance, birefringence on staining with the dye Congo Red and a cross β-sheet structure (the β-strands run perpendicular to the long axis of the filaments). The five types of amyloid-based prions are: the mammalian TSEs; [URE3], [*PSI*$^+$] and [*PIN*$^+$] of *Saccharomyces cerevisiae*; and [Het-s] of *Podospora anserina*[7–10]. These prions are based on self-propagating amyloid forms of the proteins PrP, Ure2, Sup35, Rnq1 and HET-s, respectively. PrP^C or PrP-sen, the normal form of PrP, is a non-essential glycosylphosphatidylinositol-anchored cell-surface protein that, on formation of the prion form PrP^{Sc} or PrP-res in TSE-infected animals, becomes cytotoxic — specifically to cells producing PrP^C. The precise aggregated form of PrP that is responsible for this cytotoxicity is unclear, and it may be distinct from the heritable species[11]. Ure2p is a soluble cytoplasmic negative regulator of enzymes and transporters that are needed for utilization of poor nitrogen sources[12]. In its prion form, Ure2p is ineffective for nitrogen regulation, and the enzymes and transporters that are needed for nitrogen regulation are inappropriately expressed[5]. Sup35p is a subunit of the translation termination factor. In amyloid form, Sup35p is inactive and premature termination codons (nonsense mutations) are read through at higher than normal efficiency, allowing synthesis of the full-length protein[13]. Rnq1p has no known cellular function, but it promotes formation of the [*PSI*$^+$] and [URE3] prions in its amyloid form[14]. HET-s is part of the heterokaryon incompatibility system of the filamentous fungus *P. anserina*, allowing converging colonies to distinguish self from non-self. Interestingly, it only works properly if it is in the amyloid form, and so it is a prion that has a normal function in the organism[15]. One non-amyloid-based prion in yeast, called [β], is an enzyme that has an active form (the prion, in this case) that is necessary for activation of its inactive precursor, namely vacuolar protease B[16]. A new potential prion in *P. anserina*, called [C], seems to be a self-activating mitogen-activated protein kinase kinase kinase[17].

Therefore, a prion may either be amyloid-based or not, and the prion form may be toxic or the active form of the protein, or simply inactive for the normal function of the protein (Fig. 1). Amyloid-based prions have many common features at the molecular level, and are believed to be models for the widespread human amyloid diseases, such as Alzheimer's disease (Aβ), Parkinson's disease (α-synuclein), type II diabetes (amylin/IAPP), amyotrophic lateral sclerosis (superoxide dismutase) and others.

Three faces of a prion: propagation, induction and formation

The known prions are chromosomally encoded proteins that can undergo a change such that the altered form has acquired the ability to change the unaltered form into the same altered form. Different experimental approaches measure different features of a prion protein or fragment thereof. Prion formation (or generation) is the ability of protein sequence A (possibly overproduced) to form a self-propagating prion by itself, independent of the ability of the prion to propagate to a protein of a different sequence. In a prion-induction experiment, a normal (A) or variant (A*) sequence is overexpressed, ideally transiently, in a strain that is constitutively expressing A, and the frequency of *de novo* generation of a prion of sequence A is measured.

When a prion form of protein sequence A is introduced into a strain expressing only the mutant sequence A*, the formation of the prion in the recipient strain indicates that A* can propagate the prion from A. Inability to do so is analogous to a 'species barrier'; for example, hamster scrapie cannot infect mice. These three

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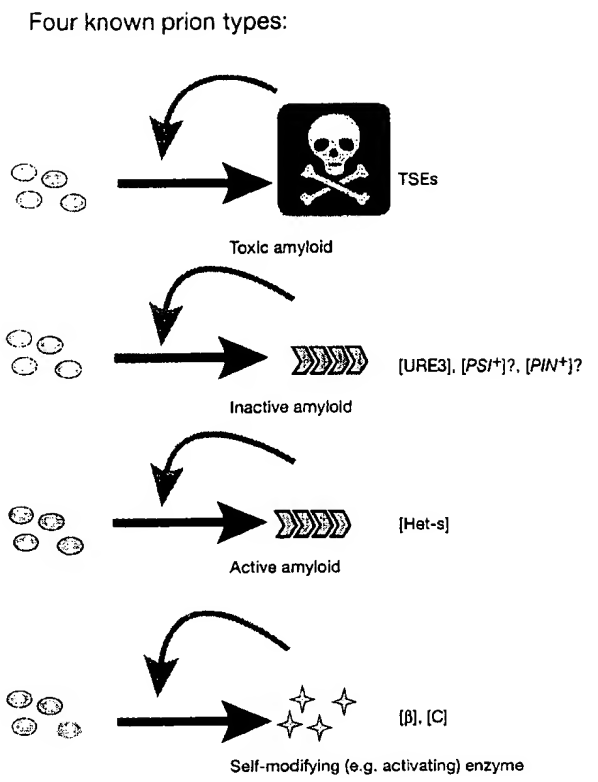

Figure 1 Classification of prions into four types. Prions may be distinguished by whether they involve amyloid or not and what the effect of the prion form is on the host: toxic (transmissible spongiform encephalopathies, TSEs), simple lack of function ([URE3], [*PSI*⁻]) or positive function for the cell ([Het-s]). Rnq1p function is unclear, so classification of [*PIN*⁺] is tentative. Green indicates the active form of the protein, whereas orange shows the inactive form.

experimental approaches can give markedly different results, and in doing so shed light on the prion phenomenon.

Prion domains

For the TSEs, [URE3], [*PSI*⁺] and [Het-s], a limited part of the corresponding protein is necessary and sufficient to transmit the prion *in vivo* and for amyloid formation *in vitro*[18–25] (Fig. 2). For Ure2p, the remainder of the molecule is the functional domain, whereas the remainder of Sup35p is composed of a highly charged middle region and the carboxy-terminal functional domain. The prion domains of PrP, Ure2p and of HET-s constitute the protease-resistant cores of the amyloids that are formed *in vivo* or *in vitro*[18–21,23–26]. Overproduction of the Ure2 or Sup35 prion domain also induces the generation of new [URE3] or [*PSI*⁺] prions, respectively. Amyloid that is formed *in vitro* from a recombinant prion domain of HET-s[27], Sup35p[28,29] or Ure2p[30] is sufficient to infect the respective hosts with [Het-s], [*PSI*⁺] or [URE3]. Attempts to generate infectious recombinant PrP *in vitro* under 'protein-only' conditions have been met with limited success[31].

Single amino-acid changes can block prion propagation

Single amino-acid changes in the Sup35 prion domain can block propagation of the wild-type sequence of [*PSI*⁺][32,33], but at least one such mutant Sup35p can also itself become a prion[34,35]. Similarly, infectious or spontaneous CJD is rarely observed in patients heterozygous for the polymorphic residue 129 of PrP, indicating a stringent requirement for sequence identity to support prion propagation[36]. Here again, PrP of either sequence can be a prion, but apparently neither can efficiently add to the amyloid that is formed by the other. Similarly, the brains of scrapie-infected hamsters are not detectably infectious for mice (the 'species barrier'). This effect is a result of sequence differences between hamster and mouse PrP and is determined by a mere 5 residues in transgenic mice[37]. However, in tissue culture infected with scrapie, a single residue (PrP 138) is sufficient to determine this barrier[38]. Expression of hamster PrP slows mouse scrapie[39], and fragments of Ure2p can eliminate the [URE3] prion[40]. This interference with prion propagation by similar molecules resembles the poisoning of crystal growth by molecules similar to those being crystallized, a parallel which is extended below.

Role of octapeptide repeats of Sup35p and PrP

Both PrP and Sup35p have octapeptide repeats in their amino-terminal regions. The familial octapeptide repeat expansions of PrP were the first demonstrated genetic link of PrP with CJD[41]. Deletion of the Sup35 repeats impairs propagation of [*PSI*⁺][20] and inserting additional repeats increases the frequency with which [*PSI*⁺] arises, an interesting parallel with the mammalian system[42]. The repeat domain also stabilizes an artificial polyQ-based prion[43]. However, whereas the octapeptide repeats are within the prion domain of Sup35p[20], they can be deleted from PrP without preventing TSE propagation[44]. In addition, the PrP repeats are not within the region that becomes protease-resistant in PrP-res[45]. Molecules of Sup35p that have one or several repeats deleted still interact with the [*PSI*⁺] aggregates, but do not propagate that interaction well[43,46], perhaps because the aggregates are not efficiently divided to make new seeds (see ref. 47 and below).

Shuffled Ure2p prion domains all form amyloid and prions

No single amino-acid changes have been shown to block [URE3] formation, although deletion of asparagine runs in the Ure2p prion domain markedly reduces prion-inducing ability[48], and a combination of two amino-acid changes in the prion domain slightly reduces prion-inducing ability[49]. To test whether there are any amino-acid sequence requirements for the Ure2p prion domain (residues 1–89), the sequence was randomized (leaving amino-acid content unchanged) and used to replace the normal prion domain in the chromosome. Unexpectedly, all five proteins with shuffled prion domains could be prions *in vivo* and form amyloid *in vitro*[50]. A similar result was obtained for five shuffled Sup35 prion domains[51]. This implies that, at least for the Ure2p and Sup35p prion domains, amino-acid composition and not amino-acid sequence determines prion and amyloid formation, and the repeat sequences are not needed for Hsp104 action. In these experiments, each shuffled molecule was allowed to form its own prions. These prion-formation experiments are in contrast to the prion-propagation experiments (see above) in which single amino-acid changes may block transmission of a prion from molecules having the normal sequence. Indeed, propagation of prions from one shuffled sequence to another or to the normal Ure2p sequence is inefficient[51]. Amyloid filaments are like linear crystals: many different molecules can each form crystals, but usually crystals of one molecule will not readily incorporate a different molecule. However, not all amyloid structures can be randomized.

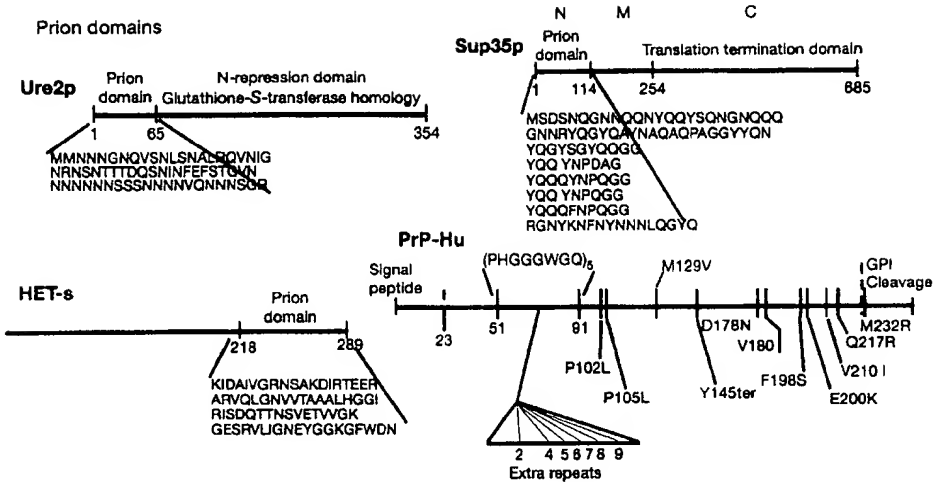

Figure 2 Prion domains. The prion-determining regions of Ure2p and Sup35p are Q/N-rich whereas Rnq1p is rich in N and Q residues through most of its length. the HET-s prion domain is *C*-terminal and not Q/N-rich. the Prp prion domain is less well-defined, but inclues at least residues 90 – 144. Mutations in PrP causing inherited CJD are shown. GPI, glycosylphosphatidylinositol.

Scrambling Aβ10–35 produces a peptide that does not form amyloid *in vitro* under conditions that allow amyloid formation by the unscrambled peptide[52].

Prion-forming ability of randomized sequences suggests parallel β-sheet structure

In an anti-parallel β-sheet or β-helix structure, it is generally non-identical residues that are hydrogen-bonded with each other (Fig. 3). These must be in some sense either complementary (plus with minus charge, large with small) or similar (hydrophobic with another hydrophobic, hydrogen-bonding between side chains). Shuffling the sequence is likely to disrupt complementary pairs, hydrogen-bonding pairs or hydrophobic pairs, and prevent prion formation.

By contrast, an in-register parallel β-sheet has identical residues paired. If the main interaction is between identical residues, it should be relatively sequence-independent (Fig. 3). Pairing of charged residues would be unfavourable in an in-register parallel structure, but no worse in the shuffled sequence. The hydrogen-bonding between Q or N residues (Perutz's 'polar zipper'[53]) would be preserved by shuffling a parallel structure, and hydrophobic residues would remain paired, but groups of hydrophobic residues would probably be broken up by shuffling either the parallel or anti-parallel structure. Therefore, a prion domain that is immune to shuffling is likely to be one with a parallel in-register β-sheet structure in the amyloid form.

Corresponding experiments with PrP have not been performed because the spontaneous rate of prion formation is too low to be experimentally measured. However, a parallel β-sheet structure would also provide an explanation for the, often extremely stringent, requirement for sequence identity for transmission or propagation of prions. Prion propagation (and amyloid growth, in general) is highly specific, with other nearby proteins not being recruited into the filaments, which are actually relatively pure (linear) crystals. Whereas [*PIN*$^+$] is an amyloid of Rnq1 that may, indeed, prime Sup35p or Ure2p polymerization to form [*PSI*$^+$] or [URE3][14,54–56], respectively, these are rare events. In strains with established [*PSI*$^+$] and [*PIN*$^+$] prions, the Sup35p and Rnq1p aggregates frequently do not colocalize, indicating that the proteins form independent filaments[55]. Furthermore, single amino-acid mutations can be sufficient to create a species barrier, which implies a parallel in-register structure, as the rough complementarity of an anti-parallel structure should be able to accommodate a single amino-acid difference in ~100 residues. Prion propagation therefore resembles crystal growth: similar but non-identical molecules can poison prion (or crystal) propagation.

Indeed, our proposal was stimulated by solid-state nuclear magnetic resonance (NMR) evidence of a parallel in-register β-sheet structure for amyloid filaments formed of Aβ1–40 (refs 57–59), and for a fragment of the Ure2 prion domain, residues 10–39 (ref. 60). Electron paramagnetic resonance spectroscopy also supports a parallel β-sheet structure for islet amyloid polypeptide (IAPP, amylin) and for α-synuclein[61,62], although designed peptides or fragments form antiparallel amyloids[63], and a parallel β-helix structure has been proposed for PrP^{Sc} (ref. 64).

There is a variety of conflicting evidence regarding the structure of Sup35p amyloid filaments. X-ray diffraction studies of dried fibres of the prion domain suggest a cross-β structure[65,66]. As discussed above, the fact that shuffling the Sup35 prion domain does not abrogate the ability to form prions indicates a parallel in-register β-sheet structure. Supporting this suggestion, a small peptide from Sup35p was recently shown by X-ray diffraction to form an in-register parallel β-sheet[67]. By contrast, another study using pyrene-labelled Sup35p to probe the structure of filaments led the authors to propose a β-helix model with head-to-head and tail-to-tail contact between the monomers[68]. However, such a model seems inconsistent with the observations of polar Sup35p amyloid fibres[69,70]. Further, Sup35 fragments that lack the 'tail' region (residues 91–106) can add to preexisting aggregates and deleting residues 98–112, including most of the tail, has no effect on [*PSI*$^+$][43,46]. Clearly, further direct structural studies will be necessary to resolve these issues.

Interactions of prion domains and the remainder of the molecule

Deletions in the C-terminal functional parts of Ure2p result in a 100-fold increase in [URE3] prion formation, a result that we suggested reflects stabilization of the prion domain in the non-prion form by interaction with the remainder of the molecule[18]. However, no interactions between the Ure2 prion domain and its C-terminal functional

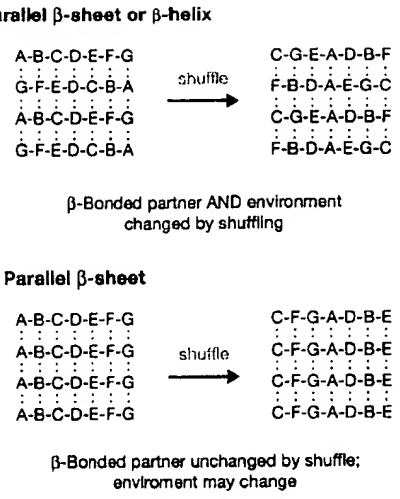

Figure 3 Sequence-independent prion formation argues for parallel β-sheet structure. In an anti-parallel β-sheet or a β-helix, hydrogen-bonded residues are nearly always different, but complementary (upper panel). Shuffling such a sequence should eliminate the complementarity and thus the ability to make the structure. In a parallel in-register β-sheet, identical residues are paired (lower panel). Shuffling, in this case should have little impact on the ability to form this structure. Therefore, if a prion domain can be shuffled and generally remains able to form prions, it probably has a parallel in-register β-sheet structure.

domain could be found by two-hybrid, surface plasmon resonance or protease experiments[71]. Moreover, NMR studies indicate that the Ure2 prion domain is largely unstructured in the native form[71]. Amyloid formation, which converts the prion domain from being unstructured to a tight β-sheet, does not inactivate fused enzymes[72] or prevent glutathione binding[73] or glutathione oxidase activity[74] by the normal Ure2 C-terminal domain. It is clear that filaments are rich in β-sheet[19,75,76] and are formed by interactions between the N termini of different molecules[18,19,26,72]. In summary, data to date argue against the interaction of prion domains and C-terminal domains.

Similarly, deletions of the Sup35p functional domain result in >1,000-fold increases in prion formation[77]. In HET-s, a single amino-acid residue outside the amyloid-forming region can determine whether the protein forms a prion, and deletion of the part that does not form amyloid increases prion and amyloid formation[25]. The core of HET-s amyloid (residues 218–289) includes a native unstructured region (residues 228–298) and a structured segment (residues 218–227)[25].

Mutations producing inherited CJD are scattered throughout the PrP molecule, but only a few of these (E200K, P101L, D178N) are known to be infectious for animals[78]. The β-sheet part of PrP^{Sc} is estimated to include residues 90–120, which are unstructured in PrP^{C} (the normal form), and also (an as yet poorly defined) part of the structured part of PrP^{C} (refs 79–81).

Chaperone-catalysed disaggregation produces new prion seeds

Whether in animals or fungi, the generation of a single amyloid filament could not become a disease or produce a phenotype unless the filament was cleaved to form new growing ends to seed new filaments. Therefore, yeast prions require a critical concentration of the disaggregating chaperone Hsp104 (refs 82–84), the function of which seems to be to break large filaments into many smaller ones, thereby producing seeds[85–88]. Hsp104 cooperates with Hsp40 and Hsp70 chaperones[89], and these have also been implicated in the prion propagation process[84,90–93]. Biochemical data suggesting that Hsp104 can, alone, disaggregate Sup35p amyloid[94] has recently been contested[95].

Mutations in the prion domain can critically affect the ability of the amyloid formed to be fragmented by the chaperone system. For example, Sup35Δ22–69, which lacks part of the Q-rich region and some of the octapeptide repeats, cannot easily propagate [*PSI*$^{+}$] from normal Sup35p[20]. However, under selection conditions, it can be a prion itself[87]. Although this [*PSI*$^{+}$]$^{\Delta 22-69}$ is unstable compared with the usual [*PSI*$^{+}$], it is stabilized by modest overexpression of Hsp104, which is accompanied by a decreased size of the Sup35Δ22–69 aggregates. Apparently, amyloid of Sup35Δ22–69 is less susceptible to fragmentation by the Hsp104 apparatus, and the resulting larger aggregates are less likely to be distributed to daughter cells.

Replacing the Sup35p N/Q-rich octapeptide repeats with repeats found in the Sup35p of the yeasts *Yarowia lipolytica* and *Debaryomyces hansenii* produced proteins that could still become prions[96]. Remarkably, neither prion required Hsp104 for its propagation. The authors suggest that the smaller amyloid fragments that are produced naturally by these hybrid proteins may relieve the requirement for breaking up filaments by Hsp104 *in vivo*. Alternatively, they suggest that there may be another Hsp104-independent seed-cleavage system[96].

The broader lesson from these studies is that the probability of an amyloidogenic protein becoming a prion may depend crucially on its interactions with chaperones. Relatively little is known about the effects of chaperones on mammalian prions, indicating that this will be an important area for future study.

Prion strains and protein sequences

Even with isogenic hosts, mammalian prions with different characteristics can be recognized based on incubation times, symptoms and tissue distribution of spongiform change[97]. Strains also vary in the way in which they cross the species barrier. Extensive data now support the notion that different strains have distinct amyloid conformations in mammals and in yeast[98]. Collinge has proposed that each PrP sequence can adopt a certain range of amyloid conformations, and that the species barrier is simply a reflection of the overlap or lack of overlap between the possible conformers of the PrP of the two species[99]. Therefore, a prion strain that happens to represent a conformer that is common to the PrP of various species will have a lower species barrier. This seems to be the case for bovine spongiform encephalopathy.

Why are so few amyloids infectious?

In vitro amyloid formation is generally an autocatalysed phenomenon. The sigmoid shape of amyloid formed versus time shows this, as does the elimination of the sigmoid shape by seeding the reaction with a small amount of the amyloid form. The *in vitro* autocatalysis and seeding indicate that the same phenomenon should occur in disease states. However, attempts to show an infectious aspect to amyloid diseases has met with limited success except for the TSEs.

One possible explanation is that the interactions of the amyloid-forming protein with other cellular components alters its behaviour from that observed in the purified state. Interacting proteins might stabilize the native form and make conversion to amyloid energetically unfavourable.

Intra- and extra-cellular spaces are packed with macromolecules and molecular crowding effects can drive amyloid formation, as the amyloid occupies less space than the soluble form[100]. In yeast, various chaperones can either promote prion propagation and generation or eliminate prions. Their dominant role is probably not unique to yeast. Amyloids may be degraded, but degradation intermediates of the normal protein might promote prion formation, as fragments of Ure2p, Sup35p and HET-s induce prion formation far more efficiently than the corresponding intact protein (see above). Prion propagation in any organism must require amyloid-filament fragmentation, a function that is carried out in yeast by Hsp104 and its cooperating chaperones.

Although all of the above factors must influence amyloid accumulation, the intrinsic properties of the amyloid-forming protein must also affect the process. Some peptides are sufficiently inclined to form amyloid that their mere production is the rate-limiting step, without the need for a seed. For others, the energy barrier to amyloid formation may be sufficient that it will rarely occur without a seed. Studies of prion domains have been central to unravelling the mysteries of prions and have shed new light on the many human amyloid diseases, some of which are widespread and growing in our ageing population. □

COMPETING FINANCIAL INTERESTS
The authors declare that they have no competing financial interests.

1. Griffith, J.S. Self-replication and scrapie. *Nature* **215**, 1043–1044 (1967).
2. Prusiner, S.B. Novel proteinaceous infectious particles cause scrapie. *Science* **216**, 136–144 (1982).
3. Tuite, M.F. & Koloteva-Levin, N. Propagating prions in fungi and mammals. *Mol. Cell* **14**, 541–552 (2004).
4. Chernoff, Y.O. Amyloidogenic domains, prions and structural inheritance: rudiments of early life or recent acquisition? *Curr. Opin. Chem. Biol.* **8**, 665–671 (2004).
5. Wickner, R.B. *et al.* Prions: proteins as genes and infectious entities. *Genes Dev.* **18**, 470–485 (2004).
6. Aguzzi, A. & Polymenidou, M. Mammalian prion biology: one century of evolving concepts. *Cell* **116**, 313–327 (2004).
7. Prusiner, S.B. Prions. *Proc. Natl Acad. Sci. USA* **95**, 13363–13383 (1998).
8. Wickner, R.B. [URE3] as an altered *URE2* protein: evidence for a prion analog in *S. cerevisiae*. *Science* **264**, 566–569 (1994).
9. Derkatch, I.L., Chernoff, Y.O., Kushnirov, V.V., Inge-Vechtomov, S.G. & Liebman, S.W. Genesis and variability of *[PSI+]* prion factors in *Saccharomyces cerevisiae*. *Genetics* **144**, 1375–1386 (1996).
10. Coustou, V., Deleu, C., Saupe, S. & Begueret, J. The protein product of the *het-s* heterokaryon incompatibility gene of the fungus *Podospora anserina* behaves as a prion analog. *Proc. Natl Acad. Sci. USA* **94**, 9773–9778 (1997).
11. Caughey, B. & Lansbury, P.T. Protofibrils, pores, fibrils, and neurodegeneration: separating the responsible prion aggregates from innocent bystanders. *Annu. Rev. Neurosci.* **26**, 267–298 (2003).
12. Magasanik, B. & Kaiser, C.A. Nitrogen regulation in *Saccharomyces cerevisiae*. *Gene* **290**, 1–18 (2002).
13. Liebman, S.W. & Derkatch, I.L. The yeast *[PSI+]* prion: making sense out of nonsense. *J. Biol. Chem.* **274**, 1181–1184 (1999).
14. Derkatch, I.L., Bradley, M.E., Hong, J.Y. & Liebman, S.W. Prions affect the appearance of other prions: the story of *[PIN]*. *Cell* **106**, 171–182 (2001).
15. Saupe, S.J. Molecular genetics of heterokaryon incompatibility in filamentous ascomycetes. *Microbiol. Mol. Biol. Rev.* **64**, 489–502 (2000).
16. Roberts, B.T. & Wickner, R.B. A class of prions that propagate via covalent auto-activation. *Genes Dev.* **17**, 2083–2087 (2003).
17. Kicka, S. & Silar, P. PaASK1, a mitogen-activated protein kinase kinase kinase that controls cell degeneration and cell differentiation in *Podospora anserina*. *Genetics* **166**, 1241–1252 (2004).
18. Masison, D.C. & Wickner, R.B. Prion-inducing domain of yeast Ure2p and protease resistance of Ure2p in prion-containing cells. *Science* **270**, 93–95 (1995).
19. Taylor, K.L., Cheng, N., Williams, R.W., Steven, A.C. & Wickner, R.B. Prion domain initiation of amyloid formation *in vitro* from native Ure2p. *Science* **283**, 1339–1343 (1999).
20. TerAvanesyan, A., Dagkesamanskaya, A.R., Kushnirov, V.V. & Smirnov, V.N. The *SUP35* omnipotent suppressor gene is involved in the maintenance of the non-Mendelian determinant [psi+] in the yeast *Saccharomyces cerevisiae*. *Genetics* **137**, 671–676 (1994).
21. King, C.-Y. *et al.* Prion-inducing domain 2–114 of yeast Sup35 protein transforms *in vitro* into amyloid-like filaments. *Proc. Natl Acad. Sci. USA* **94**, 6618–6622 (1997).
22. Glover, J.R. *et al.* Self-seeded fibers formed by Sup35, the protein determinant of *[PSI+]*, a heritable prion-like factor of *S. cerevisiae*. *Cell* **89**, 811–819 (1997).
23. Bolton, D.C., McKinley, M.P. & Prusiner, S.B. Identification of a protein that purifies with the scrapie prion. *Science* **218**, 1309–1311 (1982).
24. Fischer, M. *et al.* Prion protein (PrP) with amino-terminal deletions restoring susceptibility of PrP knockout mice to scrapie. *EMBO J.* **15**, 1255–1264 (1996).
25. Balguerie, A. *et al.* Domain organization and structure-function relationship of the HET-s prion protein of *Podospora anserina*. *EMBO J.* **22**, 2071–2081 (2003).
26. Baxa, U. *et al.* Architecture of Ure2p prion filaments: the N-terminal domain forms a central core fiber. *J. Biol. Chem.* **278**, 43717–43727 (2003).
27. Maddelein, M.L., Dos Reis, S., Duvezin-Caubet, S., Coulary-Salin, B. & Saupe, S.J. Amyloid aggregates of the HET-s prion protein are infectious. *Proc. Natl Acad. Sci. USA* **99**, 7402–7407 (2002).
28. King, C.Y. & Diaz-Avalos, R. Protein-only transmission of three yeast prion strains. *Nature* **428**, 319–323 (2004).
29. Tanaka, M., Chien, P., Naber, N., Cooke, R. & Weissman, J.S. Conformational variations in an infectious protein determine prion strain differences. *Nature* **428**, 323–328 (2004).
30. Brachmann, A., Baxa, U. & Wickner, R.B. Prion generation *in vitro*: amyloid of Ure2p is infectious. *EMBO J.* **24**, 3082–3092 (2005).
31. Legname, G. *et al.* Synthetic mammalian prions. *Science* **305**, 673–676 (2004).
32. Doel, S.M., McCready, S.J., Nierras, C.R. & Cox, B.S. The dominant *PNM2*- mutation which eliminates the [PSI] factor of *Saccharomyces cerevisiae* is the result of a missense mutation in the *SUP35* gene. *Genetics* **137**, 659–670 (1994).
33. DePace, A.H., Santoso, A., Hillner, P. & Weissman, J.S. A critical role for amino-terminal glutamine/asparagine repeats in the formation and propagation of a yeast prion. *Cell* **93**, 1241–1252 (1998).
34. Kochneva-Pervukhova, N.V. *et al.* Mechanism of inhibition of Ψ+ prion determinant propagation by a mutation of the N-terminus of the yeast Sup35 protein. *EMBO J.* **17**, 5805–5810 (1998).
35. Derkatch, I.L., Bradley, M.E., Zhou, P. & Liebman, S.W. The *PNM2* mutation in the prion protein domain of SUP35 has distinct effects on different variants of the *[PSI+]* prion in yeast. *Curr. Genet.* **35**, 59–67 (1999).
36. Mead, S. *et al.* Balancing selection at the prion protein gene consistent with prehistoric kurulike epidemics. *Science* **300**, 640–643 (2003).
37. Scott, M. *et al.* Propagation of prions with artificial properties in transgenic mice expressing chimeric PrP genes. *Cell* **73**, 979–988 (1993).
38. Priola, S.A., Caughey, B., Race, R.E. & Chesebro, B. Heterologous PrP molecules interfere with accumulation of protease-resistant PrP in scrapie-infected murine neuroblastoma cells. *J. Virol.* **68**, 4873–4878 (1994).
39. Prusiner, S.B. *et al.* Transgenic studies implicate interactions between homologous PrP isoforms in scrapie prion replication. *Cell* **63**, 673–686 (1990).
40. Edskes, H.K., Gray, V.T. & Wickner, R.B. The [URE3] prion is an aggregated form of Ure2p that can be cured by overexpression of Ure2p fragments. *Proc. Natl Acad. Sci. USA* **96**, 1498–1503 (1999).
41. Owen, F. *et al.* Insertion in prion protein gene in familial Creutzfeldt–Jakob disease. *Lancet* **1**, 51–52 (1989).
42. Liu, J.J. & Lindquist, S. Oligopeptide-repeat expansions modulate 'protein-only' inheritance in yeast. *Nature* **400**, 573–576 (1999).
43. Osherovich, L.Z., Cox, B.S., Tuite, M.F. & Weissman, J.S. Dissection and design of yeast prions. *PLoS Biol* 2, E86 (2004).
44. Flechsig, E. *et al.* Prion protein devoid of the octapeptide repeat region restores susceptibility to scrapie in PrP knockout mice. *Neuron* **27**, 399–408 (2000).
45. Prusiner, S.B., Groth, D.F., Bolton, D.C., Kent, S.B. & Hood, L.E. Purification and structural studies of a major scrapie prion protein. *Cell* **38**, 127–134 (1984).
46. Parham, S.N., Resende, C.G. & Tuite, M.F. Oligopeptide repeats in the yeast protein Sup35p stabilize intermolecular prion interactions. *EMBO J.* **20**, 2111–2119 (2001).
47. Borchsenius, A.S., Wegrzyn, R.D., Newnam, G.P., Inge-Vechtomov, S.G. & Chernoff, Y.O. Yeast prion protein derivative defective in aggregate shearing and production of new 'seeds'. *EMBO J.* **20**, 6683–6691 (2001).
48. Maddelein, M.-L. & Wickner, R.B. Two prion-inducing regions of Ure2p are non-overlapping. *Mol. Cell. Biol.* **19**, 4516–4524 (1999).
49. Fernandez-Bellot, E., Guillemet, E. & Cullin, C. The yeast prion [URE3] can be greatly induced by a functional mutated URE2 allele. *EMBO J.* **19**, 3215–3222 (2000).
50. Ross, E.D., Baxa, U. & Wickner, R.B. Scrambled prion domains form prions and amyloid. *Mol. Cell. Biol.* **24**, 7206–7213 (2004).
51. Ross, E.D., Edskes, H.K., Terry, M.J. & Wickner, R.B. Primary sequence independence for prion formation. *Proc. Natl Acad. Sci. USA* **102**, 12825–12830 (2005).
52. Antzutkin, O.N., Leapman, R.D., Balbach, J.J. & Tycko, R. Supramolecular structural constraints on Alzheimer's β-amyloid fibrils from electron microscopy and solid-state nuclear magnetic resonance. *Biochemistry* **41**, 15436–15450 (2002).
53. Perutz, M.F., Johnson, T., Suzuki, M. & Finch, J.T. Glutamine repeats as polar zippers: their possible role in inherited neurodegenerative diseases. *Proc. Natl Acad. Sci. USA* **91**, 5355–5358 (1994).
54. Bradley, M.E., Edskes, H.K., Hong, J.Y., Wickner, R.B. & Liebman, S.W. Interactions among prions and prion "strains" in yeast. *Proc. Natl Acad. Sci. USA* **99 (Suppl. 4)**, 16392–16399 (2002).
55. Derkatch, I.L. *et al.* Effects of Q/N-rich, polyQ, and non-polyQ amyloids on the *de novo* formation of the [PSI+] prion in yeast and aggregation of Sup35 *in vitro*. *Proc. Natl Acad. Sci. USA* **101**, 12934–12939 (2004).
56. Salnikova, A.B., Kryndushkin, D.S., Smirnov, V.N., Kushnirov, V.V. & Ter-Avanesyan, M.D. Nonsense suppression in yeast cells overproducing Sup35 (eRF3) is caused by its non-heritable amyloids. *J. Biol. Chem.* **280**, 8808–8812 (2005).
57. Benzinger, T.L. *et al.* Propagating structure of Alzheimer's β-amyloid(10–35) is parallel

β-sheet with residues in exact register. *Proc. Natl Acad. Sci. USA* **95**, 13407–13412 (1998).
58. Antzutkin, O.N. *et al.* Multiple quantum solid-state NMR indicates a parallel, not antiparallel, organization of β-sheets in Alzheimer's β-amyloid fibrils. *Proc. Natl Acad. Sci. USA* **97**, 13045–13050 (2000).
59. Tycko, R. Insights into the amyloid folding problem from solid-state NMR. *Biochemistry* **42**, 3151–3159 (2003).
60. Chan, J.C.C., Oyler, N.A., Yau, W.-M. & Tycko, R. Parallel β-sheets and polar zippers in amyloid fibrils formed by residues 10–39 of the yeast prion protein Ure2p. *Biochemistry* **44**, 10669–10680 (2005).
61. Jayasinghe, S.A. & Langen, R. Identifying structural features of fibrillar islet amyloid polypeptide using site-directed spin labeling. *J. Biol. Chem.* **279**, 48420–48425 (2004).
62. Der-Sarkissian, A., Jao, C.C., Chen, J. & Langen, R. Structural organization of α-synuclein fibrils studied by site-directed spin labeling. *J. Biol. Chem.* **278**, 37530–37535 (2003).
63. Balbach, J.J. *et al.* Amyloid fibril formation by Aβ16–22, a seven-residue fragment of the Alzheimer's β-amyloid peptide, and structural characterization by solid state NMR. *Biochemistry* **39**, 13748–13759 (2000).
64. Govaerts, C., Wille, H., Prusiner, S.B. & Cohen, F.E. Evidence for assembly of prions with left-handed β-helices into trimers. *Proc. Natl Acad. Sci. USA* **101**, 8342–8347 (2004).
65. Serio, T.R. *et al.* Nucleated conformational conversion and the replication of conformational information by a prion determinant. *Science* **289**, 1317–1321 (2000).
66. Kishimoto, A. *et al.* β-Helix is a likely core structure of yeast prion Sup35 amyloid fibers. *Biochem. Biophys. Res. Commun.* **315**, 739–745 (2004).
67. Nelson, R. *et al.* Structure of the cross-β spine of amyloid-like fibrils. *Nature* **435**, 773–778 (2005).
68. Krishnan, R. & Lindquist, S. Structural insights into a yeast prion illuminate nucleation and strain diversity. *Nature* **435**, 765–772 (2005).
69. DePace, A.H. & Weissman, J.S. Origins and kinetic consequences of diversity in Sup35 yeast prion fibers. *Nature Struct. Biol.* **9**, 389–396 (2002).
70. Inoue, Y., Kishimoto, A., Hirao, J., Yoshida, M. & Taguchi, H. Strong growth polarity of yeast prion fiber revealed by single fiber imaging. *J. Biol. Chem.* **276**, 35227–35230 (2001).
71. Pierce, M.M., Baxa, U., Steven, A.C., Bax, A. & Wickner, R.B. Is the prion domain of soluble Ure2p unstructured? *Biochemistry* **44**, 321–328 (2005).
72. Baxa, U., Speransky, V., Steven, A.C. & Wickner, R.B. Mechanism of inactivation on prion conversion of the *Saccharomyces cerevisiae* Ure2 protein. *Proc. Natl Acad. Sci. USA* **99**, 5253–5260 (2002).
73. Bousset, L., Belrhali, H., Melki, R. & Morera, S. Crystal structures of the yeast prion Ure2p functional region in complex with glutathione and related compounds. *Biochemistry* **40**, 13564–13573 (2001).
74. Bai, M., Zhou, J.M. & Perrett, S. The yeast prion protein Ure2 shows glutathione peroxidase activity in both native and fibrillar forms. *J. Biol. Chem.* **279**, 50025–50030 (2004).
75. Schlumpberger, M. *et al.* The prion domain of yeast Ure2p induces autocatalytic formation of amyloid fibers by a recombinant fusion protein. *Protein Sci.* **9**, 440–451 (2000).
76. Baxa, U. *et al.* Filaments of the Ure2p prion protein have a cross-β core structure. *J. Struct. Biol.* **150**, 170–179 (2005).
77. Kochneva-Pervukhova, N.V., Poznyakovski, A.I., Smirnov, V.N. & Ter-Avanesyan, M.D. C-terminal truncation of the Sup35 protein increases the frequency of *de novo* generation of a prion-based [*PSI*$^+$] determinant in *Saccharmyces cerevisiae*. *Curr. Genet.* **34**, 146–151 (1998).
78. Brown, P. *et al.* Human spongiform encephalopathy: the National Institutes of Health series of 300 cases of experimentally transmitted disease. *Ann. Neurol.* **35**, 513–529 (1994).
79. Locht, C., Chesebro, B., Race, R. & Keith, J.M. Molecular cloning and complete sequence of prion protein cDNA from mouse brain infected with the scrapie agent. *Proc. Natl Acad. Sci. USA* **83**, 6372–6376 (1986).
80. Basler, K. *et al.* Scrapie and cellular PrP isoforms are encoded by the same chromosomal locus. *Cell* **46**, 417–428 (1986).
81. Riek, R., Hornemann, S., Wider, G., Glockshuber, R. & Wuthrich, K. NMR characterization of the full-length recombinant murine prion protein, mPrP(23–231). *FEBS Lett.* **413**, 282–288 (1997).
82. Chernoff, Y.O., Lindquist, S.L., Ono, B.-I., Inge-Vechtomov, S.G. & Liebman, S.W. Role of the chaperone protein Hsp104 in propagation of the yeast prion-like factor [psi$^+$]. *Science* **268**, 880–884 (1995).
83. Derkatch, I.L., Bradley, M.E., Zhou, P., Chernoff, Y.O. & Liebman, S.W. Genetic and environmental factors affecting the *de novo* appearance of the [*PSI*+] prion in *Saccharomyces cerevisiae*. *Genetics* **147**, 507–519 (1997).
84. Moriyama, H., Edskes, H.K. & Wickner, R.B. [URE3] prion propagation in *Saccharomyces cerevisiae*: requirement for chaperone Hsp104 and curing by overexpressed chaperone Ydj1p. *Mol. Cell. Biol.* **20**, 8916–8922 (2000).
85. Ness, F., Ferreira, P., Cox, B.S. & Tuite, M.F. Guanidine hydrochloride inhibits the generation of prion "seeds" but not prion protein aggregation in yeast. *Mol. Cell. Biol.* **22**, 5593–5605 (2002).
86. Jung, G., Jones, G. & Masison, D.C. Amino acid residue 184 of yeast Hsp104 chaperone is critical for prion-curing by guanidine, prion propagation, and thermotolerance. *Proc. Natl Acad. Sci. USA* **99**, 9936–9941 (2002).
87. Paushkin, S.V., Kushnirov, V.V., Smirnov, V.N. & Ter-Avanesyan, M.D. Propagation of the yeast prion-like [*psi*$^+$] determinant is mediated by oligomerization of the *SUP35*-encoded polypeptide chain release factor. *EMBO J.* **15**, 3127–3134 (1996).
88. Wegrzyn, R.D., Bapat, K., Newnam, G.P., Zink, A.D. & Chernoff, Y.O. Mechanism of prion loss after Hsp104 inactivation in yeast. *Mol. Cell. Biol.* **21**, 4656–4669 (2001).
89. Glover, J.R. & Lindquist, S. Hsp104, Hsp70, and Hsp40: a novel chaperone system that rescues previously aggregated proteins. *Cell* **94**, 73–82 (1998).
90. Newnam, G.P., Wegrzyn, R.D., Lindquist, S.L. & Chernoff, Y.O. Antagonistic interactions between yeast chaperones Hsp104 and Hsp70 in prion curing. *Mol. Cell. Biol.* **19**, 1325–1333 (1999).
91. Kushnirov, V.V., Kryndushkin, D.S., Boguta, M., Smirnov, V.N. & Ter-Avanesyan, M.D. Chaperones that cure yeast artificial [PSI$^+$] and their prion-specific effects. *Curr. Biol.* **10**, 1443–1446 (2000).
92. Jung, G., Jones, G., Wegrzyn, R.D. & Masison, D.C. A role for cytosolic Hsp70 in yeast [PSI$^+$] prion propagation and [PSI$^+$] as a cellular stress. *Genetics* **156**, 559–570 (2000).
93. Allen, K.D. *et al.* Hsp70 chaperones as modulators of prion life cycle: novel effects of Ssa and Ssb on the *Saccharomyces cerevisiae* prion [PSI$^+$]. *Genetics* **169**, 1227–1242 (2005).
94. Shorter, J. & Lindquist, S. Hsp104 catalyzes formation and elimination of self-replicating Sup35 prion conformers. *Science* **304**, 1793–1797 (2004).
95. Inoue, Y., Taguchi, H., Kishimoto, A. & Yoshida, M. Hsp104 binds to yeast Sup35 prion fiber but needs other factor(s) to sever it. *J. Biol. Chem.* **279**, 52319–52323 (2004).
96. Crist, C.G., Nakayashiki, T., Kurahashi, H. & Nakamura, Y. [*PHI*$^+$], a novel Sup35-prion variant propagated with non-Gln/Asn oligopeptide repeats in the absence of the chaperone protein Hsp104. *Genes Cells* **8**, 603–618 (2003).
97. Bruce, M.E., McConnell, I., Fraser, H. & Dickinson, A.G. The disease characteristics of different strains of scrapie in *Sinc* congenic mouse lines: implications for the nature of the agent and host control of pathogenesis. *J. Gen. Virol.* **72**, 595–603 (1991).
98. Chien, P., Weissman, J.S. & DePace, A.H. Emerging principles of conformation-based prion inheritance. *Annu. Rev. Biochem.* **73**, 617–656 (2004).
99. Collinge, J. Variant Creutzfeldt–Jakob disease. *Lancet* **354**, 317–323 (1999).
100. Hatters, D.M., Minton, A.P. & Howlett, G.J. Macromolecular crowding accelerates amyloid formation by human apolipoprotein C-II. *J. Biol. Chem.* **277**, 7824–7830 (2002).